

Page 47, line 12:

In another embodiment of the invention, the altered or mutated forms of structure (I) are obtained by substituting at least one amino acid with a non-conservative amino acid. Those of skill in the art will recognize that such substitutions should not substantially alter the amphipathic and/or structural properties of the helix discussed, supra. Thus, in certain instances it may be desirable to substitute one or more pairs of amino acids so as to preserve the net properties of the helix. Further guidance for selecting appropriate amino acid substitutions is provided by the peptide sequences listed in ~~TABLE X~~ TABLE IX (see, Section 8.3, infra).

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In still another preferred embodiment, the ApoA-I agonists are selected from the group of peptides set forth below:

peptide 191	PVLDLLRELLEELKQKLK*	(SEQ ID NO:191);
peptide 192	PVLDLFKELLEELKQKLK*	(SEQ ID NO:192);
peptide 193	PVLDLFRELLEELKQKLK*	(SEQ ID NO:193);
peptide 194	PVLELFRELLEELKQKLK*	(SEQ ID NO:194);
peptide 195	PVLELFKELLEELKQKLK*	(SEQ ID NO:195);
peptide 196	PVLDLFRELLEELKNKLK*	(SEQ ID NO:196);
peptide 197	PLLDLFRELLEELKQKLK*	(SEQ ID NO:197);
peptide 198	GVLDLFRELLEELKQKLK*	(SEQ ID NO:198);
peptide 199	PVLDLFRELWEELKQKLK*	(SEQ ID NO:199);
peptide 200	NVLDLFRELLEELKQKLK*	(SEQ ID NO:200);
peptide 201	PLLDLFKELLEELKQKLK*	(SEQ ID NO:201);
peptide 202	PALELFKDLLEELRQKLR*	(SEQ ID NO:202);
peptide 203	AVLDLFRELLEELKQKLK*	(SEQ ID NO:203);
peptide 204	PVLDFFRELLEELKQKLK*	(SEQ ID NO:204);
peptide 205	PVLDLFREWLEELKQKLK*	(SEQ ID NO:205);
peptide 206	PLLELLKELLEELKQKLK*	(SEQ ID NO:206);
peptide 207	PVLELLKELLEELKQKLK*	(SEQ ID NO:207);

peptide 208	PALELFKDLLEELRQRLK*	(SEQ ID NO:208);
peptide 209	PVLDLFDRELLNELLQKLK	(SEQ ID NO:209);
peptide 210	PVLDLFDRELLEELKQKLK	(SEQ ID NO:210);
peptide 211	PVLDLFDRELLEELOQOLO*	(SEQ ID NO:211);
peptide 212	PVLDLFDRELLEELOQOLK*	(SEQ ID NO:212);
peptide 213	PALELFKDLLEEFQRRLK*	(SEQ ID NO:213);
peptide 214	pVLDLFDRELLEELKQKLK*	(SEQ ID NO:214);
peptide 215	PVLDLFDRELLEEWKQKLK*	(SEQ ID NO:215);
<del>peptide 229</del>	PVLELFDRELLLEDLQKKLK	<del>(SEQ ID NO:229);</del>
<u>peptide 230</u>		<u>(SEQ ID NO: 230);</u>
<del>peptide 230</del>	PVLDLFDRELLEKLEQKLK	<del>(SEQ ID NO:230);</del>
<u>peptide 231</u>		<u>(SEQ ID NO:231);</u>
<del>peptide 231</del>	PLLELFDKELLEELKQKLK*	<del>(SEQ ID NO:231);</del>
<u>peptide 232</u>		<u>(SEQ ID NO:232);</u>

in either the N- and/or C-terminal blocked or unblocked forms.

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In a final preferred embodiment, the ApoA-I agonists are not any of the peptides listed in ~~TABLE X~~ TABLE IX (Section 8.3, infra) which exhibit an LCAT activation activity of less than 38% as compared with native human ApoA-I.

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#### 6. EXAMPLE: SYNTHESIS OF PEPTIDE AGONISTS OF ApoA-I

The peptides described in ~~TABLE X~~ TABLE IX (Section 8.3, infra) were synthesized and characterized as described in the subsections below. The peptides were also analyzed structurally and functionally as described in Sections 7 and 8, infra.

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#### 6.2 SYNTHESIS OF PEPTIDE AMIDES

Where indicated in ~~TABLE X~~ TABLE IX (Section 8.3, infra), peptide amides were

synthesized using a Rink amide resin containing the Fmoc-Rink amide handle 4-(2', 4'-dimethylphenyl)-Fmoc-phenoxyethyl (Rink, 1987, Tetrahedron Lett. 28: 3787-3790) and the synthesis protocols described in section 6.1, supra.

### 6.3 SYNTHESIS OF N-TERMINAL ACYLATED PEPTIDES

Where indicated in ~~TABLE X~~ TABLE IX (section 8.3, infra), N-terminal acylated forms of the peptides were prepared by exposing the resin-bound peptide prepared as described in section 6.1 or 6.2, supra, to an appropriate acylating agent.

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After background ~~subtraction~~, subtraction, spectra were converted to molar ellipticity ( $\theta$ ) per residue in  $\text{deg. cm}^{-2} \text{ dmol}^{-1}$ . The peptide concentration was determined by amino acid analysis and also by absorption spectrometry on a Perkin Elmer Lambda 17 UV/Visible spectrophotometer when the peptide contained a chromophore (~~tryptophane~~, tryptophan, dansyl, ~~naphthylalanine~~ naphthylalanine).

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### 7.1.3 RESULTS

The degree of helicity (%) of the free, unbound peptides (free), the peptide-SUV complexes (SUVs), the peptide-micelle complexes (mics) and the peptide-TFE solution (TFE) are reported in ~~TABLE X~~, TABLE IX, section 8.3, infra.

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Referring to ~~TABLE X~~, TABLE IX, section 8.3, infra, it can be seen that those peptides which exhibit a high degree of LCAT activation ( $\geq 38\%$ ) generally possess significant  $\alpha$ -helical structure in the presence of lipids ( $\geq 60\%$  helical structure in the case of unblocked peptides containing 22 or more amino acids or blocked peptides containing 18 or fewer amino acids;  $\geq 40\%$  helical structure in the case of unblocked peptides containing 18 or fewer amino acids), whereas peptides which exhibit little or no LCAT activation possess little  $\alpha$ -helical structure. However, in some instances, peptides which contain significant  $\alpha$ -helical structure in the presence of lipids do not exhibit significant LCAT activation. As a consequence, the ability of the core peptides of the invention to adopt an  $\alpha$ -helical structure in

the presence of lipids is considered a critical feature of the core peptides of the invention, as the ability to form an  $\alpha$ -helix in the presence of lipids appears to be a prerequisite for LCAT activation.

## 7.2 FLUORESCENCE SPECTROSCOPY

The lipid binding properties of the peptides synthesized in Section 6, *supra*, were tested by fluorescence measurements with labeled peptides, in the present case ~~Tryptophane~~ tryptophan (Trp or W) or ~~Naphthylalanine~~ naphthylalanine (Nal). The fluorescence spectra were recorded on a Fluoromax from Spex (Jobin-Yvon) equipped with a Xenon lamp of 150W, two monochromators (excitation and emission), a photomultiplier R-928 for detection sensitive in the red up to 850 nm and a thermoelectric magnetic stirred cell holder. Quartz Suprasil cuvettes were used for measurements in the micromolar concentration range. A device of variable slits (from 0.4 to 5 nm) allows modulation of the incident and emitted intensities according to the concentration of peptide used. The reported values are in general the average of between 2 to 4 spectra. The peptide concentration is determined by absorption spectrometry on a Philips PU 8800 using the absorption band of the Trp ( $\epsilon_{280\text{ nm}} = 5,550\text{ M}^{-1}\text{cm}^{-1}$  in Tris buffer) or the Nal ( $\epsilon_{224\text{ nm}} = 92,770\text{ M}^{-1}\text{cm}^{-1}$  in methanol).

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The fluorescence spectra of the peptides were characterized by the wavelength at their maximum of fluorescence emission and by their quantum yield compared to NATA in the case of peptides labeled with a ~~tryptophane~~ tryptophan. The process of binding to lipids was analyzed by calculating the shift of the wavelength at the maximum of fluorescence emission, ( $\lambda_{\text{max}}$ ), and the variation of the relative fluorescence intensity of emission versus the lipid concentration. The relative fluorescence intensity is defined as the following ratio:  $(I - I_0)_{\lambda_{\text{max}}} / I_{0\lambda_{\text{max}}}$ .  $I$  and  $I_0$  are both measured at the ( $\lambda_{\text{max}}$ ) corresponding to the initial free state of the peptide, *i.e.*, without lipids.  $I$  is the intensity at a defined lipid to peptide ratio and  $I_0$  is the same parameter measured in absence of lipids. The absence of these variations is relevant ~~of~~ to the absence of interactions of the peptides with the lipids.

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In buffer at a concentration of 2  $\mu\text{M}$ , the maximum of the ~~tryptophane~~ tryptophan fluorescence emission ( $\lambda_{\text{max}}$ ) of peptide **199** (SEQ ID NO:199) is 348 nm. This corresponds

to a ~~tryptophane~~ tryptophan which is relatively exposed to the aqueous environment when compared to NATA ( $\lambda_{\text{max}} = 350 \text{ nm}$ ). Peptide 199 (SEQ ID NO:199) binds very effectively to EPC/Chol (20:1) small unilamellar vesicles as demonstrated by the burying of the ~~tryptophane~~ tryptophan (the wavelength for the [tryptophane] tryptophan maximum fluorescence emission shifts from 348 nm to 325 nm) and the high fluorescence intensity exaltation (See Table VII). The burying of the ~~tryptophane~~ tryptophan residue is maximal for a lipid to peptide molar ratio of about 100.

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## 8.2 PURIFICATION OF LCAT

For the LCAT purification, dextran sulfate/ $\text{Mg}^{2+}$  treatment of human plasma is used to obtain lipoprotein deficient serum (LPDS), which is sequentially chromatographed on Phenylsepharose, Affigelblue, ConcanavalinA sepharose and anti-ApoA-I affinity chromatography, as summarized for a representative purification in ~~TABLE IX~~, TABLE VIII, below:

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~~TABLE IX~~ TABLE VIII

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## 8.3 RESULTS

The results of the LCAT activation assay are presented in ~~TABLE X~~, TABLE IX, infra.

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~~TABLE X~~ TABLE IX

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In ~~TABLE X~~, TABLE IX, \* indicates peptides that are N-terminal acetylated and C-terminal amidated; † indicates peptides that are N-terminal dansylated; sp indicates peptides that exhibited solubility problems under the experimental conditions; X is Aib; Z is Nal; O is Orn; He (%) designates percent helicity; mics designates micelles; and ~ indicates deleted amino acids.